Isolated beef heart mitochondria, swollen in 100 mM Na⁺ or K⁺ nitrate, contract and extrude the accumulated salt in a respiration-dependent reaction. The reaction is more rapid and extensive in Na⁺ than in K⁺ nitrate. Contraction is inhibited by uncouplers and by reagents, such as valinomycin and gramicidin, which increase transmembrane permeability to cations. The rate and extent of contraction are strongly dependent on pH, with lower rates and decreased efficiency under the alkaline conditions which favor high electrophoretic permeability to cations. The contraction reaction is characterized by high rates of respiration which return to controlled values when ion extrusion is complete. The rate of contraction is directly proportional to the rate of respiration at neutral pH. Estimates of the efficiency of contraction show that, depending on the pH, anywhere from 2 to 6 Na⁺ ions may be extruded per succinate oxidized. Both the rate and the efficiency of contraction are increased by the addition of nigericin at pH values above 7.2 in Na⁺ nitrate and an even greater stimulation of the reaction in K⁺ produced by addition of nigericin when the pH is above 7.0. Contraction is inhibited as extramitochondrial Na⁺ or K⁺ is increased and, at unfavorable salt concentrations, the reaction is stimulated by decreased pH. Less net H⁺ extrusion is detected by a glass electrode during contraction than when respiration is initiated in unswollen mitochondria. Contraction is also activated by mersalyl at concentrations just over that required to abolish phosphate oxidant transport. The results are consistent with the suggestion that respiration-dependent contraction is an osmotic response to the extrusion of accumulated cations on an endogenous cation/H⁺ exchanger.

Isolated mitochondria can utilize metabolic energy to bring about either a net uptake or a net extrusion of salts depending upon the experimental conditions imposed (see Refs. 5 and 6 for reviews). Net ion uptake (swelling) occurs when mitochondria respire in K⁺ acetate, for example, whereas net ion extrusion (contraction) is observed when respiration is initiated in mitochondria swollen in Na⁺ or K⁺ nitrate (2-6). The available evidence suggests that cation uptake in the acetate medium results from conversion of the protonmotive force of respiration (7) to an acetate gradient through penetration of free acetic acid present in equilibrium with the acetate ion (5, 7, 8). Under these conditions the electrophoretic entrance of cation in response to the negative interior potential (ΔΨ) would then result in accumulation of an ion pair and osmotic swelling. In contrast, when mitochondria have been swollen in nitrate salts, the permeant nitrate anion would minimize any membrane potential and the protonmotive force would be expressed as a transmembrane pH gradient (ΔpH). In two recent preliminary communications (3, 4) we have outlined a series of experiments that support the view that the respiration-dependent contraction of mitochondria swollen in nitrate salts is an osmotic response which depends on (a) respiration-dependent production of ΔpH, (b) utilization of ΔpH via exchange of exterior H⁺ for internal Na⁺ or K⁺ on an endogenous cation/H⁺ exchanger, and (c) electrophoretic efflux of nitrate and osmotic contraction in response to the decreased positive charge of the interior. The model shown in Fig. 1 summarizes this series of events. The present communication provides a more complete account of these studies and further develops the concept that mitochondrial K⁺ levels are maintained by a regulated interplay between an electrochemical cation entry mechanism and an exchanger-mediated efflux pathway (1, 5, 6, 9, 10).

MATERIALS AND METHODS

Nagarse beef heart mitochondria were prepared as previously described (9). Swelling and contraction were monitored at 546 nm using an Eppendorf photometer equipped with a water-jacketed circular cuvette and magnetic stirrer. Respiration and pH were recorded simultaneously with a YSI oxygen electrode and a Thomas 4858 combination glass electrode. The total water and sucrose-permeable water space of centrifuged pellets of mitochondria were estimated as previously described (11) and the cation content was determined by atomic absorption spectroscopy of perchloric acid extracts.

RESULTS

Passive Swelling of Heart Mitochondria in Nitrate Salts — Nonrespiring heart mitochondria swell only slowly at 25°C when suspended in Na⁺ or K⁺ nitrate (100 mM) at neutral pH...
Energy-dependent Contraction of Mitochondria

Fig. 1 (left). Osmotic model for respiration-dependent contraction of mitochondria swollen in Na+ nitrate. The activity of the electron transport system (ETS) results in ejection of H+ to the cytosol side of the inner membrane (T) and an increase in matrix pH (pHm). Matrix Na+(Na)m, exchanged out for external H+ on an endogenous cation/H+ exchanger (E). The incoming H+ is neutralized by matrix OH− and, as a result, the internal positive charge is decreased. The decreased positive charge of the interior causes efflux of the permeant nitrate anion and osmotic contraction (net flow of water from the matrix side, m, to the cytosol side of the membrane). Efflux on the exchanger is opposed by a passive inward leak of cations through a pathway which becomes significant at elevated pH (cation uniport). Passive swelling increases considerably, however, when the pH is increased (Fig. 2A) and when the reaction is carried out at temperatures above 30° (Fig. 2B). Swelling at pH 8.3 and 35° has been found to provide a conveniently rapid rate of swelling with minimal alteration of membrane properties. Swelling under these conditions permits the study of respiration-dependent contraction in the absence of reagents, such as valinomycin, which alter membrane permeability and which have been used to produce passive swelling in several previous studies of the contraction reaction (5, 13–16). Passive swelling is more rapid in Na+ than in K+ nitrate at identical pH and temperature (Fig. 2, A and B), but much of this differential is due to a more extended lag time before swelling commences in the K+ medium (Fig. 2C). Swelling in Li+ nitrate is much slower than that in Na+ or K+ under these conditions.

The permeability changes induced by elevated pH seem to be reversible, since passive swelling virtually ceases when the pH is returned to 7.1 by addition of nitric acid (Fig. 3, Trace b). Mitochondria swollen in Na+ or K+ nitrate also retain a low permeability to sucrose. Passive swelling is strongly inhibited by addition of sucrose to the suspending medium under the conditions of Fig. 2 and a rapid contraction is seen when sucrose is added to swollen, nonrespiring mitochondria at pH 8.5 (Fig. 3, Trace a). At pH 7.1, addition of the same amount of sucrose produces a much slower contraction which is accelerated by reagents which increase cation permeability (i.e. gramicidin, Fig. 3, Trace b). These results suggest that accumulated Na+ nitrate is readily extruded when mitochondria are contracted by sucrose osmotic pressure at the elevated pH, but that a transmembrane permeability to Na+ restricts the sucrose-dependent contraction at neutral pH.

The rate of passive swelling in Na+ or K+ nitrate at any given pH is strongly inhibited by low concentrations of Mg2+ nitrate and accelerated by gramicidin (see Fig. 3 of Ref. 3).

Cation Content of Swollen Mitochondria – Nonrespiring heart mitochondria suspended in Na+ nitrate at pH 7 retain considerable K+ at 37° (decrease from 103 to 58 nmol of K+/mg of protein in 2 min). When the pH is increased to 8.3 to initiate passive swelling, however, the loss of endogenous K+ is rapid and virtually complete (decrease to 2 nmol of K+/mg). There is also a decline in endogenous Mg2+ under conditions of passive swelling in Na+ nitrate (decrease from 32 to 24 nmol of Mg2+/(mg). Mitochondria isolated by centrifugation after increasing periods of swelling at pH 8.3 show increased Na+ content consistent with osmotic swelling (Fig. 4). Under these conditions the total water in the mitochondrial pellet increases by 1.3 μl/mg of protein for each 0.1 A546 unit. When allowance is made for a slight increase in osmotic permeable space in these pellets (Fig. 4B), the increase in matrix water volume (11) becomes approximately 1.0 μl/mg/0.1 A546. The Na+ analysis of these mitochondrial pellets shows an increase...
of 128 nmol of Na\(^+\)/mg for each 0.1 A\(_{460}\) unit which corresponds well to the value predicted (130 nmol of Na\(^+\)/mg) for an increase of 1.3 \(\mu\)l of solvent containing 100 mM Na\(^+\). When corrected for the Na\(^+\) content of the sucrose-permeable space, the Na\(^+\) and tritiated water data both indicate that a change of 1.3 \(\mu\)l of solvent containing 100 mM Na\(^+\) equals 128 nmol of Na\(^+\)/mg of protein. Analysis of mitochondria which have been contracted by washing in sucrose at 0\(^\circ\)C shows that Na\(^+\) is extruded and matrix water decreased to values of 105 nmol of Na\(^+\) and 1.0 \(\mu\)l of water/mg of protein.

**Respiration-dependent Contraction of Mitochondria Swollen in Nitrate Salts** — Addition of succinate to initiate respiration in the mitochondria swollen in NaNO\(_3\) results in contraction and extrusion of accumulated ions (Fig. 5A). The contraction is more rapid and extensive at lower pH values than the pH 8.3 used for the swelling phase of the reaction. Contraction is abolished by antimycin and other inhibitors of respiration. There is little re-swelling when the respiring mitochondria reach the anaerobic point at pH 7.1, but rapid swelling does occur at pH 8.3 when oxygen is exhausted (Fig. 5A). Contraction at pH 8.3 is accompanied by an elevated rate of respiration (0.29 \(\mu\)atom of O\(_2\)/min \(\cdot\) mg \(^{-1}\)) typical of State 3 (I7) or uncoupled beef heart mitochondria and respiration remains linear throughout the contraction. Contraction at pH 7.1 is accompanied by a burst of elevated respiration which returns to a controlled rate (0.10 \(\mu\)atom \(\cdot\) min \(\cdot\) mg \(^{-1}\)) after the contraction phase has been completed (Fig. 5A).

Analysis of mitochondria which have been contracted by succinate respiration is complicated by re-swelling which occurs during centrifugation. These mitochondria show greatly decreased matrix water and decreased Na\(^+\) content, however, and the matrix Na\(^+\) concentration remains at approximately 100 mM Na\(^+\).

Contraction in the 100 mM K\(^+\) nitrate medium is less rapid and extensive than that in the Na\(^+\) salt at any given pH (compare Fig. 5, A and B) and respiration in the K\(^+\) salt is poorly controlled during contraction (Fig. 5B). Contraction in both Na\(^+\) and K\(^+\) is sensitive to uncouplers such as dinitrophenol and CCP\(^\prime\) (Fig. 5B). Contraction is also diminished by ouabain which increases transmembrane permeability to Na\(^+\) or K\(^+\), such as gramicidin (see Fig. 4 in Ref. 3) or valinomycin in the K\(^+\) medium (Fig. 5B). In addition, contraction is strongly inhibited by anions which support net, respiration-dependent ion accumulation (5), such as acetate (2.5 mM produces 90% inhibition) or phosphate (data not shown).

The rate of energy-dependent contraction is directly proportional to the rate of respiration, since when succinate oxidation at pH 7.45 is controlled by simultaneous addition of the competitive inhibitor malonate, a plot of the rate of contraction versus rate of oxidation produces a straight line (Fig. 6). The contraction can also be supported by exogenous ATP. In this case, a rapid rate of contraction is accompanied by elevated ATPase activity, but both contraction and ATPase decline rapidly. The decline is probably due to production of the inhibitory phosphate anion. Low rates of ATPase are seen when the extrusion of Na\(^+\) is complete (under the conditions of Fig. 5A).

**Activation of Energy-dependent Contraction by Nigericin** — Respiration-dependent contraction is strongly activated by the addition of Tris/succinate (2.5 mM) of appropriate pH. The respiration rate (\(\mu\)atoms of O\(_2\)/min \(\cdot\) mg \(^{-1}\)) is given in parentheses. The concentration of other additions was as follows: antimycin, 1.0 \(\mu\)g/mg of protein; nigericin, 2 \(\times\) 10\(^{-7}\) M; valinomycin, 1 \(\times\) 10\(^{-4}\) M; CCP (m-chlorocarboxylylidephenylhydrazone), 1 \(\times\) 10\(^{-4}\) M. In each case these reagents were added just prior to the succinate.
nigericin (optimal response at 2 x 10^{-7} M) under appropriate conditions and the contraction in K^+ is affected to a greater extent than that in Na^+ (4). Contraction in the presence of nigericin remains sensitive to antimycin, uncouplers, and valinomycin (4) and, like the reaction in the absence of this reagent, is proportional to the rate of respiration. In the presence of nigericin, respiration in the K^+ nitrate medium shows the control phenomenon described for the reaction in Na^+ (Fig. 5B, oxygen records). When respiration-dependent contraction is initiated by addition of succinate of varied pH and the rate of contraction compared with the pH maintained during the contraction, a pH optimum of about 6.8 is obtained for contraction in NaNO_3 (Fig. 7). The pH profile for K^+ contraction under these conditions shows a broad peak at about pH 7.0. Addition of nigericin (2 x 10^{-7} M) just prior to the succinate results in a marked enhancement of respiration-dependent contraction in K^+, especially at more alkaline pH, so that the pH optimum for contraction shifts to 7.5 (Fig. 7). The contraction in Na^+ is strongly stimulated at alkaline pH under these conditions (pH optimum 7.6), but below pH 7.2 the rate of contraction is depressed by nigericin (Fig. 7). Some of this inhibition of contraction by nigericin seems to be the result of decreased uptake of succinate by the nigericin-treated mitochondria, since the reaction is characterized by low rates of succinate respiration (Table I) and contraction supported by ascorbate-TMPD does not reflect such a marked inhibition by nigericin at pH 6.8 (Table I).

**Efficiency of Contraction Reaction**—The plots relating the Na^+ content of the matrix to A_{Na} (Fig. 4B) permit a calculation of the efficiency of the contraction reaction based on the Na^+ extruded per oxygen consumed (Na^+/O).

In the Na^+ medium, the low rate of contraction at pH 8.3 results in a lower efficiency than that at 7.1, even though the respiration rates are identical during the contraction phase (see Fig. 4 of Ref. 3 for a plot of the decline in efficiency of contraction in Na^+ nitrate with increasing pH). Contraction is less efficient in K^+ than in Na^+ nitrate, since the rate of contraction is lower and respiration is nearly the same in the two salts (Fig. 5, A versus B). As we have noted, efficiency of respiration-dependent contraction is also increased by Mg^{2+}, a cation which decreases passive swelling in Na^+ and K^+ nitrate and, by inference, transmembrane permeability to the cations (3).
The contraction reaction is surprisingly efficient, showing values of Na⁺/O of 6 to 7 for succinate oxidation at the optimal pH of 6.85 (Table I). The efficiency of contraction is lower at higher pH (Table I and Fig. 6). Addition of nigericin increases both the rate of contraction and the efficiency at pH 7.6, the optimal pH for contraction in the presence of the exogenous exchanger (Table I). At an intermediate pH, such as 7.1, the rate of contraction with nigericin present is actually lower in the absence of this reagent but, since the respiration is affected to an even greater extent, the efficiency remains high (Na⁺/O of about 6). The efficiency of respiration-dependent extrusion of Na⁺ supported by succinate is about twice that supported by ascorbate-TMPD (Table I, Experiment 3).

Succinate-dependent H⁺ Ejection during Contraction—When respiration is initiated by addition of succinate to rotenone-treated mitochondria, a brief burst of H⁺ ejection occurs with an apparent limiting stoichiometry of nearly 8 H⁺/O consumed (18, 19). If the respiration-dependent contraction of swollen mitochondria results from Na⁺ extrusion on a Na⁺/H⁺ exchanger (cf. Fig. 1), then one would predict that less H⁺ ejection would be visible to the glass electrode during the concentration reaction. Comparison of H⁺ ejection during contraction with that seen with unswollen mitochondria under otherwise identical conditions (Fig. 8), shows that this prediction is verified. Unswollen mitochondria eject about 20 neq of H⁺·mg⁻¹ at a rate of 170 neq of H⁺·min⁻¹·mg⁻¹ when respiration is initiated with succinate (Fig. 8B). Swollen mitochondria eject protons at a rapid rate for about 2 s following addition of succinate (Fig. 8A), but as contraction commences the rate of H⁺ ejection slows to a very low rate (approximately <10 neq·min⁻¹·mg⁻¹). This less extensive H⁺ ejection occurs during contraction despite the fact that steady state respiration is nearly 3 times faster under these conditions than in the absence of contraction (270 versus 110 natriom of O₂·min⁻¹·mg⁻¹). The alkaline shift upon addition of uncoupler indicates that a ΔpH of 80 neq·mg⁻¹ has established in mitochondria respiring in the absence of contraction (Fig. 8B) as opposed to 14 neq of H⁺·mg⁻¹ in the succinate-contracted mitochondria (Fig. 8A). These studies suggest that there is an initial outflow of H⁺ from the swollen mitochondrion, but that as a ΔpH is established, the ejected H⁺ is exchanged into the mitochondrion for internal Na⁺ (see Fig. 1) and contraction commences. In the presence of nigericin, the ejection of H⁺ is even less extensive than in the absence of this exogenous exchanger (data not presented).

Effect of Concentration of Na⁺ or K⁺ Nitrate on Respiration-Dependent Contraction—Only small differences in the rate of passive swelling are observed when mitochondria are suspended in Na⁺ or K⁺ nitrate at concentrations higher than 100 mM (Fig. 2C). However, the respiration-dependent contraction reaction at pH 7.1 is strongly inhibited as the concentration of Na⁺ nitrate is increased and is virtually eliminated at 100 mM (Fig. 2A). Contraction in K⁺ nitrate does not occur at 140 mM or above and the reaction in this salt is optimal at about 60 mM K⁺ nitrate (Fig. 2B). At 60 mM K⁺ nitrate the respiration rate returns to a low or controlled level after the contraction phase is complete as is seen for the reaction in Na⁺. This controlled respiration is not seen with higher concentrations of the K⁺ salt, however (Fig. 2B). Respiration declines in the Na⁺ medium as the contraction becomes inhibited by increased external Na⁺ (Fig. 2A).

Effect of pH on Contraction in High Concentrations of Na⁺ Nitrate—The inhibitory effects of high external salt concentration on the contraction reaction can be relieved to some extent by decreasing the pH. The low rate of succinate-supported contraction seen in 150 mM Na⁺ nitrate at pH 7.5,.
than 20 nmol/mg gives nearly the same initial rates of function of pH in a medium of 150 mM Na+ nitrate at 35°. The effects are enhanced by mersalyl with a maximum effect at just over 12 mM Na+ succinate of protein (Fig. 11B). Since the increase in succinate-dependent contraction is accompanied by a sharp decrease in absorbance from B divided by the rate of respiration in microatoms of O2.mg-1.min-1.

In agreement with the findings of Azzone et al. (14, 20), the mechanism for respiration-dependent contraction and, in addition, strongly suggest that the reaction depends on the activity of an endogenous cation/H+ exchanger as shown in Fig. 1. The presence of a cation/H+ exchanger (Na+ > K+) in the mitochondrial membrane was postulated by Mitchell and Moyle (22) on the basis of studies of the rate of decay of respiration-induced pH gradients. This exchange activity has been invoked to explain the passive swelling of mitochondria in Na+ acetate (5, 23, 24), the energy-dependent uptake of cations by SMP (24, 25), the rapid efflux of accumulated H+ from SMP at the anaerobic point (26), and Na+/K+ discrimination in beevericin-treated mitochondria (27). It has also been postulated that such an exchange could function to extrude excess cations at the expense of a portion of the protonmotive force of respiration (28). This concept can be extended (as detailed in Fig. 1) to account for net respiration-dependent ion extrusion and contraction of swollen mitochondria. Evidence in support of this model can be summarized as follows:

**Rate and Efficiency of Contraction Decrease with Increasing Transmembrane Permeability to Cations**—Contraction is inhibited by valinomycin (Fig. 5B) and by gramicidin (3), both of which increase cation permeability. These reagents would be expected to decrease the efficiency of contraction dependent on cation/H+ exchange (Fig. 1) by permitting the extruded cations to leak back into the matrix more readily. Contraction is also less rapid and efficient at elevated than at neutral pH (Ref. 3 and Figs. 5, 7, and 10, and Table I).
Elevated pH appears to increase transmembrane permeability to Na\(^+\) and K\(^+\) (indicated as the pH 8.3 uniport in Fig. 1) since passive swelling in nitrate (Fig. 2), thiocyanate, and other salts of permeant anions becomes significant above pH 8.3 Mg\(^{2+}\) \(\cdot\) which inhibits passive swelling in Na\(^+\) and K\(^+\) nitrate (to a greater extent than can be ascribed to its osmotic contribution), increases the efficiency of respiration-dependent contraction at high pH (3). This effect of Mg\(^{2+}\) would be consistent with decreased uniport activity in the presence of the divalent cation in the model shown in Fig. 1 (29).

The inverse relationship between transmembrane permeability to cations and efficiency of contraction argues strongly against contractile models for respiration-dependent ion extrusion by mitochondria. An analogy for this type of mechanism is provided by the sucrose-dependent osmotic contraction shown in Fig. 3, since sucrose osmotic pressure is used to "squeeze out" accumulated solutes and solvent in this protocol. In this case contraction is accelerated by increasing the transmembrane permeability to cations with either gramicidin or elevated pH and, as just discussed, the opposite holds for the respiration-dependent contraction.

**Cation and Anion Selectivity Are Consistent with Model**—Contraction is favored by permeant anions (5, 15), inhibited by permeant acids (such as acetate), and follows the same cation selectivity sequence as the passive swelling in acetate salts (4), a reaction which is presumed to depend on the endogenous cation/H\(^+\) exchanger.

*Observed Effects on Respiration, H\(^+\) Ejection, and Efficiency Are Consistent with Model*—The model shown in Fig. 1 predicts that less H\(^+\) ejection will be observed following a pulse of reductant (18) when back-exchange of H\(^+\) for Na\(^+\)\(_{\text{in}}\), is occurring than in the absence of contraction and this is the observed result (Fig. 8). The high efficiency of Na\(^+\) extrusion (limiting values of Na\(^+/\)O of 6 to 7 for succinate, Table I) is consistent with the availability of 8 H\(^+/\)succinate which recent estimates suggest as a limit (18, 19). This assumes that, under conditions of maximum efficiency of Na\(^+\) extrusion, virtually the entire available proton motive force of respiration can be channeled into Na\(^+/\)H\(^+\) exchange. The facts that the efficiency of contraction with succinate is twice that with ascorbate-TMPD (Table I) and that the rate of contraction is directly proportional to the rate of respiration are consistent with the model shown (Fig. 1), but are difficult to rationalize in terms of contractile models. The model in Fig. 1 also predicts that as ΔpH is consumed by exchange of [Na\(^+\)\(_{\text{in}}\)], for [H\(^+\)], the rate of respiration will be accelerated (7, 28) in much the same way that respiration is controlled by ADP in oxidative phosphorylation measurements (17). This accelerated respiration and the return to controlled rates of ΔpH uptake when contraction is complete are among the most striking features of the contraction reaction (Fig. 5) when it is used under conditions of low transmembrane permeability to cations.

**Contraction Is Stimulated by Exogenous Exchanger Nigericin**—In the model (Fig. 1) nigericin would provide a parallel exchange pathway to that provided by the endogenous exchanger. The proportionally greater stimulation of K\(^+\) extrusion in the presence of nigericin is in line with the cation selectivity of this antibiotic (30). The observation that respiration-dependent cation extrusion can be activated by nigericin in intact mitochondria complements previous reports that ion uptake by submitochondrial particles is enhanced by addition of this exchanger (24, 25). Since the particles have the opposite membrane orientation to that of intact mitochondria, ion uptake in SMP should correspond to ion extrusion by mitochondria. The marked upward shift of the pH optimum for contraction (Fig. 7) and the decreased rate of contraction when nigericin is added at low pH (Fig. 7 and Table I) may be explained by the relatively high pK\(_a\) of nigericin (8.45, Ref. 31) and the uncoupler-like effects of the protonated antibiotic on substrate uptake and retention. It is clear that, in the appropriate pH range, nigericin can provide remarkable enhancement of the rate of respiration-dependent contraction and that this reagent can increase the efficiency of an otherwise unfavorable reaction to a limit of 6 to 7 Na\(^+\) extruded for each succinate oxidized (Table I).

**Contraction Is Inhibited by Elevated External Salt and Stimulated by External H\(^+\)**—In terms of Fig. 1, increased [Na\(^+\)], would make net exchange of [Na\(^+\)\(_{\text{in}}\), for [H\(^+\)], less favorable, whereas increased [H\(^+\)], would favor the exchange. Both of these effects can be demonstrated (Figs. 9 and 10). Estimates of internal Na\(^+\) concentration (Fig. 4) indicate that there is little difference in [Na\(^+\)], versus [Na\(^+\)\(_{\text{in}}\)], when swelling has reached about 0.5 Amax unit. Contraction is completely dependent on respiration, so the driving force for the reaction must be provided by metabolic H\(^+\) ejection (which could produce elevated [H\(^+\)], in close proximity to the membrane exchanger). At elevated [pH], however, metabolic H\(^+\) would tend to be neutralized by the buffering power of the suspending medium. Plots of the reciprocal of the efficiency of contraction in Na\(^+\) nitrate (conditions of Fig. 10) versus the reciprocal of [H\(^+\)], are linear in the pH range from 7.2 to 7.6 and show that the reaction is inhibited competitively by increasing concentrations of Na\(^+\) nitrate (plots not shown). A number of complications, such as back-diffusion of Na\(^+\) through the uniport (Fig. 1), alternative routes for utilization of ΔpH, and other effects of pH on respiration and membrane components, limit this demonstration of the competitive relationship between [Na\(^+\)], and [H\(^+\)], to such restricted ranges of salt and pH as to be no more valuable than the plots showing a qualitative cross-dependency between the two components (i.e. Figs. 9 and 10).

It is of possible significance that external K\(^+\) concentrations in the physiological range (approximately 140 mM) are strongly inhibitory to respiration-dependent K\(^+\) efflux (Fig. 9B). This would prevent loss of mitochondrial K\(^+\) to the cytosol by the exchange pathway in situ except under conditions of extramitochondrial K\(^+\) depletion. It is also of interest that Na\(^+\) cycling does not seem to occur in respiring heart mitochondria at neutral pH, since respiration and ATPase return to low rates once the accumulated Na\(^+\) has been extruded (Figs. 5 and 9). This suggests that once Na\(^+\) is extruded, it does not leak back into the matrix. This is clearly not the case with K\(^+\), however, since the high rates of respiration associated with K\(^+\) extrusion in 100 mM or higher K\(^+\) nitrate (Fig. 9B) do not return to controlled rates, a result which suggests that considerable K\(^+\) cycling is occurring under these conditions. Recent studies of "K" influx and...
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The present studies are quite consistent with our interpretation of the 

The marked activation of ion efflux by mersalyl (Fig. 11) indicates that alternative pathways for dissipation of \( \Delta \nu \) (such as phosphate transport) normally take precedence over cation/H\(^{+}\) exchange under many conditions. Certainly mersalyl, at concentrations just over those required to eliminate anion exchange permits a remarkable increase in respiration-dependent contraction when the reaction conditions are otherwise very unfavorable.

The present studies have established that isolated heart mitochondria are capable of extruding ions and contracting in a very efficient reaction, but that the conditions necessary to demonstrate the contraction are restrictive. It is not clear whether the conditions which would permit swollen mitochondria to use respiratory energy to contract would ever arise in vivo. It seems quite possible that the cation/H\(^{+}\) exchanger has other roles in mitochondrial metabolism, such as adjustment of the \( \Delta \nu \) versus the \( \Delta \gamma \) components of protonmotive force (10, 27, 28), and that contraction in the nitrate salts is merely a response to a set of unusual conditions which emphasizes the endogenous exchange capacity. Contraction of mitochondria swollen with more physiological anions, such as phosphate (20), indicates that some degree of volume adjustment may be possible under physiological conditions, however.

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Energy-dependent contraction of swollen heart mitochondria.
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